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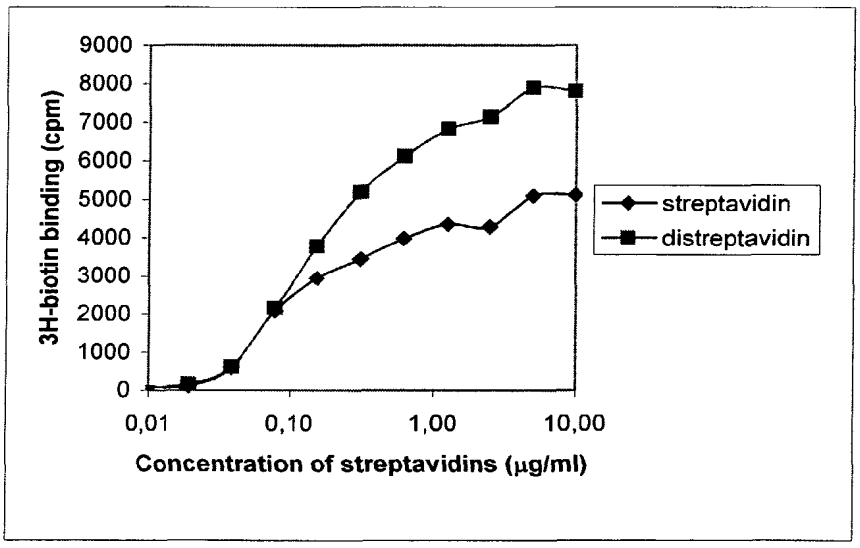
(54) Title: AVIDIN DIMERS EFFECTIVE IN INCREASING TIIE CONCENTRATION OF RADIOACTIVE BIOTIN IN PRE-TARGETED RADIOIMMUNOTHERAPY



(57) Abstract: Dimers of avidin and streptavidins (diavidins) are described wherein the linker is suberate, which in turn, is bound to different functional groups (-NH_2 o-COOH) of avidin. As compared to avidin, the diavidins have shown the ability to increase the amount of labelled biotin on the target, when used in an *in vitro*pretargeting test using supported human tenascin, the biotinylated anti-tenascin monoclonal antibody (Mab-B), avidin/diavidin, and biotin-^3H. The use of such diavidins is also described in cancer diagnosis and anticancer therapy based on the three-step pretargeted radioimmunotherapy procedure.

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Avidin dimers effective in increasing the concentration of radioactive biotin in pretargeted radioimmunotherapy

The invention described herein relates to derivatives of avidin which are useful in the diagnosis and treatment of tumours, and particularly in the so-called three-step pretargeting method.

Technical field

The invention described herein relates to modified avidins which are useful for use in human and animal diagnosis and therapy, and particularly for the diagnosis and treatment of pathological conditions such as tumours.

The invention described herein relates to the technical field of the preparation of medicaments and diagnostic means and provides compounds, methods for their preparation, methods for their use, and compositions containing them which are suitable for industrial application in the pharmaceutical field.

The invention described herein provides compounds, compositions and methods which are useful in diagnostic and therapeutic medicament, as image acquisition techniques and treatments for pathological conditions of organs and tissues.

In particular, but not exclusively, the present invention relates to the field of tumour therapy by means of radiopharmaceuticals.

Background to the invention

Tumour therapy is mainly implemented by means of the use of substances aimed at killing the tumour cells. This can be achieved with cytotoxic substances which have to enter the tumour cell in order to exert their full effect, or by means of treatment of the tumour cells with radiation with sufficient energy to kill the cell. In both cases, there is

the problem of delivering the substance as selectively as possible to the target cell, so as to avoid possible damage to the surrounding healthy cells. In the case of radiopharmaceuticals, i.e. of substances bearing radioactive portions, the problem of selectively delivering the active part (that is to say, the radioactive portion) to the tumour target, avoiding the spread of radionuclide in the body or in the healthy cells surrounding the tumour, is of particular concern.

One particularly effective method for tumour detection and therapy is described in patent EP 0 496 074. The protocol of this patent has been applied to the so-called Pretargeted Antibody-Guided Radioimmunotherapy (PAGRIT) of brain tumours. In this method, avidin is injected into the human subject, after the biotinylated anti-tenascin monoclonal antibody (Mab-B), to remove any free Mab-B, not bound to the tumour, from the bloodstream by forming complexes with it that are effectively eliminated by the liver (chase effect). An infusion of streptoavidin is then administered for the purposes of obtaining better avidination of the tumour compared to that obtainable with avidin, whose permanence in the blood is too short compared to that of streptoavidin.

Though the system has shown positive clinical responses (*Cremonesi, M. et al., 1999; Paganelli, G. et al., 1999; Paganelli, G. et al., 2001*), one major limiting factor consists in the strong immune response caused by streptoavidin *(Paganelli, G. et al., 1997*). For the purposes of overcoming these two obstacles, i.e. the high degree of immunogenicity of streptoavidin and the rapid clearance of avidin, avidins have been used which are chemically modified by covalently binding polyoxyethylene glycol (PEG) chains to avidin, with various levels of derivatisation based on the use of straight or branched PEGs of different molecular weights. Preliminary studies have revealed that, with the increase in the degree of functionalisation of avidin with PEG (hereinafter referred to as pegilation), there is an increase in the plasma half-life of avidin, a reduction in immunogenicity, and an

improvement in the specific biodistribution of the substance in relation to the tumour.

Since the ability of avidin-PEG to bind to Mab-B biotin is reduced by pegilation, the result is a reduction in the potency of the derivatives (*Chinol, M. et al., 1998*).

A solution to this problem has been proposed in patent application WO 94/23759, filed in the name of Immunomedics, where avidin multipolymers are described based on the chemical derivatisation of high-molecular-weight molecules, preferably greater than 5,000 Da, such as dextrane, proteins and polycarboxylic acids. But none of the five multimers effectively described in the patent has been characterised in terms of its potency of action in the pretargeting procedure or in other procedures.

As demonstrated in the invention described herein, the general concept of multimerisation (also including dimerisation), given in the above-mentioned patent application WO 94/237599, fails to provide complete and sufficient instructions for the average technician in finding a generic avidin multimer capable of fulfilling the necessary requirements in the application of the three-step pretargeting method. In fact, different diavidins, obtained using different bifunctional cross-linkers, though possessing the same ability to bind free biotin, differ in their potency when assayed *in vitro* in three-step pretargeting, to the extent that, in certain cases, they prove to be completely inefficacious.

This observation indicates that the multimerisation of avidin does not automatically produce useful functional products, but that biological characterisation is necessary for the choice of a potentiated molecule suitable for pretargeting.

Summary of the invention

It has now been found that by binding two molecules of avidin with a bifunctional linker, capable of binding the amino and/or carboxy groups of avidin, selected from disuccinimidyl suberate (dimer hereinafter referred to as diavidin 1) and PEG diamine with molecular weight 3400 (dimer hereinafter referred to as diavidin 2), two avidin dimers are obtained which fulfil the requisites for use in the tumour treatment method known as PAGRIT.

Thus the objects of the invention described herein are an avidin dimer in which two molecules of avidin are bound via the $-NH_2$ groups by means of a suberate and an avidin dimer in which two molecules of avidin are bound via the –COOH groups by means of polyethylene glycol with a molecular weight of 3400.

Further objects of the invention described herein are pharmaceutical and/or diagnostic compositions containing the above-mentioned diavidins.

Other objects of the present invention are the use of diavidins as medicaments or diagnostic agents for pathological conditions of organs and tissues, and particularly for the preparation of medicaments useful for the therapy or diagnosis of tumours.

These and other objects related to the present invention will be illustrated in detail here below, also by means of experimental examples.

Detailed description of the invention

As intended in the present invention, avidin means both avidin and streptavidin, but the case in which streptavidin is used as particular embodiment of the present invention, this fact will be specified.

Diavidin 1 was prepared by reacting avidin with disuccinimidyl suberate (DSS), having N-hydroxysuccinimidyl (NHS ester) as the

active ester, DSS being a homobifunctional cross-linker reactive in binding the $-NH_2$ group of avidin.

Diavidin 2 and diavidin 3 (negative control) were generated using PEG diamine ($PEG(NH_2)_2$) with a molecular weight of 3400 and polyethylene glycol-disuccinimidylpropionic acid [$PEG\ (SPA)_2$] with a molecular weight of 3400, respectively, as homobifunctional cross-linkers.

Due to the slower elimination of streptavidin compared to avidin from the circulation, distreptavidin are a particular embodiment of the present invention. The longer half-life is crucial to achieve the maximum increase in efficiency of avidins. The protocol for streptavidin cross-linking was similar to the one used for diavidin 1 production.

The pharmaceutical or diagnostic compositions according to the invention described herein contain at least one of the diavidins described here. The diavidin will be in a mixture with suitable vehicles and/or excipients commonly used in pharmacy, such as those described in "Remington's Pharmaceutical Sciences Handbook", latest edition. The compositions according to the present invention will contain an efficacious amount of diavidin.

Preferred examples of pharmaceutical compositions are those that permit parenteral and locoregional administration. Pharmaceutical compositions suitable for the purpose are solutions, suspensions, or lyophilised forms to be reconstituted at the time of use.

As regards the use of the diavidins according to the present invention, these are particularly suitable for the preparation of medicaments or diagnostic means for the diagnosis or therapy of pathological conditions of tissues, such as, for example, tumours, by means of the technique known as pretargeting with antibodies, and for this reason are also suitable for *in-vitro* pretargeting techniques. In one realisation by way

of an example, the pretargeting technique is implemented with a biotinylated anti-tenascin antibody, preferably a monoclonal antibody.

Suitable forms for the industrial application of the present invention are also kits for diagnosis or therapy, particularly the radiotherapy of tumours, such as, for example, is described in EP 0 496 074, in the study by Paganelli, Chinol *et al.* published in the *European Journal of Nuclear Medicament Vol. 26, No 4; April 1999; 348-357*, US 5.968.405 and related literature.

A further object of the invention described herein is a kit for tumour therapy or diagnosis, particularly by means of radioactivity, for example, with the pretargeting method, preferably three-step, characterised in that at least one of the components of said kit contains a diavidin. In said kit, one preferred biotinylated antibody is an anti-tenascin antibody, and even more preferably a monoclonal antibody.

The following examples further illustrate the invention.

Example 1

Diavidin 1

1 ml of avidin solution, 300 μM in PBS, pH 7.4, was mixed with 25 μl of DSS (from Pierce) 25 mM in DMS0 (avidin:DSS ratio: 1:2). The mixture was incubated for 2 hours at 0°C before blocking the reaction with 50 μl of Tris 1M, pH 8.0. The choice of the aforesaid reaction ratio was based on preliminary tests using ratios from 1:1 to 1:10.

The reaction scheme is as follows:

$$\text{Avidin -NH}_2 + \text{DSS} + \text{H}_2\text{N - Avidin}$$

$$\Downarrow$$

$$\text{Avidin – NH-CO(CH}_2)_6\text{ CONH – Avidin + 2NHS}$$

(diavidin 1)

Example 2

Diavidin 2

1 ml of avidin, 450 μM in PBS, pH 7.4, was mixed with 120 μl of PEG $(NH_2)_2$ (from Shearwater Corp.) 9 mM in H_2O and 50 μl of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide-HCl (EDAC) 260 mM in DMSO (avidin:PEG ratio: 1:2.5 approx.) and left to react for 2 hours at ambient temperature. At the end of this period 50 μl of Tris 1 M, pH 8.0, were added and the mixture was submitted to gel filtration. The avidin:PEG ratio was investigated over a range from 1:1 to 1:10 at a reaction pH from 4.0 to 8.0. The value of the PEG:avidin ratio in the purified diavidin 2 end product was 0.9, using the method described by Sims *et al.*, 1980. In brief, diavidin 2 was diluted to 300 μM in water, 250 μl of 5% $BaCl_2$ in HCl 1N were added to a volume of 1 ml, and then 250 μl of a solution prepared by mixing 1.27 g of I_2 in 100 ml of KI 2%. The mixture was incubated for 15 minutes and then the absorbance reading was taken at 535 nm. The standard curve was obtained with PEG $(NH_2)_2$.

The reaction scheme for diavidin 2 is as follows

Avidin - COOH + H_2N - PEG - NH_2 + HOOC - Avidin

⇩ EDAC

Avidin - CONH - PEG - NHCO - Avidin
(diavidin 2)

Example 3

Diavidin 3

1 ml of avidin 150 μM in PBS, pH 7.4, was mixed with 20 μl of PEG disuccinimidyl-propionate (SPA – PEG – SPA) 20 mM in H_2O (avidin:PEG ratio: 1:3.5) and left to react for 2 hours at 0°C. The reaction ratio was selected on the basis of preliminary tests conducted with ratios ranging from 1:2 to 1:10. The value of the PEG:avidin ratio in the purified dimer, as determined using the method developed by Sims *et al.*, as above, was 3:1. The reaction scheme is as follows:

Avidin-NH_2 + NHS-CO-CH_2CH_2OPEGOCH_2CH_2CONHS + H_2N-Avidin

⇩

Avidin – NHCOCH2CH2OPEGOCH2CH2CONH - Avidin
(diavidin 3)

The diavidin yield in the three reactions described in examples 1, 2 and 3 was approximately 20-30%. On increasing the amount of the three linkers in the reactions, greater final amounts of avidin oligomers were obtained (trimer, etc., not shown), with difficulties in chromatographic separation as a result. The reaction mixtures were analysed on a Superdex 200 –10/30 gel filtration column, while the purification of the products was done on a Superdex 200 –16/60 column. The chromatography profiles of the reaction mixtures for diavidin 1, 2 and 3 are shown in Figures 1 a, b and c, respectively. The molecular weights of a series of standard proteins (calibration) are indicated at the respective elution times. The calibration of the column is shown in Figure 1 d: dextrane blue (Vo), ferritin (444 KDa), aldolase (158 KDa), albumin (67 KDa), and ribonuclease (14 KDa) were used.

The purified avidin dimers are presented in Figures 1 e, f and g. The samples were separated on Superdex 200 –10/15 at a flow rate of 0.5 ml/min. (a-d) and 1 ml/min (e-g) in PBS on the Jasco HPLC system connected up to a 280 nm spectrophotometer.

Example 4

Distreptavidin

1ml of streptavidin (300 μM in PBS, pH 7.4) was mixed with 25 μl of DSS (25mM in DMSO) at an streptavidin:DSS ratio of 1:2 and incubated for 2hrs at 0°C before the reaction was quenched with 50μl 1M TRIS, pH 8.0. A total of 4 reaction conditions were tested with a ratio of streptavidin:DSS ranging from 1:1 to 1:10. We selected the above described ratio of 1:2.

The reaction scheme is analogous to the ones reported in the previous Examples.

The chromatographic profile of the crosslinking mixture at the end of the reaction for distreptavidin is shown in the figure 5a.The purified distreptavidin is shown in the figure 5b and streptavidin in 5c. The samples were analyzed on a Superdex200 10/15column at a flow of 1ml/min in PBS on a Jasco HPLC system connected to a spectrophotometer measuring the absorbance at 280nm.

Determination of the ability of diavidins to bind biotin

To compare the ability of avidin and diavidin to bind biotin the HABA (4-hydroxy-azobenzene-2'-carboxylic acid) method was used. Avidin and diavidins were all in a concentration corresponding to 3 μM of 67 KDa avidin monomer, in 0.1 M phosphate, 0.4 mM HABA at pH 7.0. Biotin dissolved in phosphate was then added to a final concentration ranging from 0 to 20 μM and the absorbance was measured at 500 nm.

The ability to bind biotin was assessed as the biotin concentration necessary to displace 50% of bound HABA.

Biotin 5 μM approx. was capable of displacing 50% of HABA both with avidin and with the three diavidins (Figure 2), from which it can be

deduced that the diavidins conserve the total number of binding sites after the cross-linking. The biotin-binding properties of diavidin are comparable to those of avidin.

In-vitro pretargeting assays

To test the ability of diavidins to increase the amount of radiolabelled biotin binding to tenascin via the biotinylated anti-tenascin monoclonal antibody (Mab-B), the *in-vitro* pretargeting assay schematically illustrated in Figure 3 was used.

In brief, a 96-well plate was adsorbed with 0.5 μg/well of human tenascin (Tn-C) for 16 hours at 4°C. After three washings with PBS and 0.1% Tween 20. the residual adsorbent sites in the wells were blocked with PBS, 2% BSA 2% and 0.1% Tween 20, for 1 hour at ambient temperature. Two biotinylated anti-tenascin monoclonal antibodies (ST2146 or ST2077) were then incubated for 2 hours in the wells, at the saturating concentration of 10 μg/ml. After washing as above, avidin or diavidin were incubated in duplicate in the wells at increasing concentrations. Lastly, a saturating amount of 5 pmol of biotin-^{3}H (1.6 TBq/mmol) was incubated for 1 hour in each well. After washings, the plate reading was taken in a β-counter. As shown in Figure 4, for the two MAbs used, diavidin 1 and diavidin 2 produce an increase in bound biotin compared to avidin; diavidin 3 shows no increase with MAb ST2146 or shows a reduction of binding ability with MAb ST2077.

As compared to avidin, diavidin 2 at the concentration of 2.5 μg/ml shows an increase in the amount of bound biotin-^{3}H by a factor of 2.1 (mean of 3 experiments) with Mab ST2077. For diavidin 1 the increase was by a factor of 1.6 (mean of 6 experiments), whereas for diavidin 3 the binding ability was lower (90%) compared to the avidin monomer.

From these experiments it can be concluded that both the length of the linker and the binding sites involved in the diavidin dimer affect the

activity of the dimer in pretargeting mediated by biotinylated antibodies.

Distreptavidin proved to be more potent them streptavidin in vitro as shown in the figure 6.

Microtiter 96 well plates were coated with 0.5 μg/well of human TnC for 16 hrs at 4 °C, washed 3 times with PBS, 0.1% Tween-20 and then blocked for unspecific binding with PBS/2%BSA/0.1%Tween-20. The biotinylated anti-TnC antibody ST2146 was added at a concentration of 10 μg/ml for 2 hrs. The wells were washed 3 times with PBS/Tween-20 and thereafter streptavidin or distreptavidin were added at the indicated concentrations. Finally, 5 pmol 3H-biotin (1.6TBq/mmol) were added, the wells incubated for 2hrs, washed and counted in a β-counter.

As shown in figure 6 distreptavidin mediates increased binding of radiolabeled biotin compared to streptavidin.

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Paganelli G., Bartolomei M., Ferrari M., Cremonesi M., Broggi G., Maira G., Sturiale C., Grana C., Prisco G., Gatti M., Caliceti P., Chinol M. Pre-targeted locoregional radioimmunotherapy with ^{90}Y-biotin in glioma patients: Phase I study and preliminary therapeutic results. Cancer Biother & Radiopharm 16(3):227-235, 2001.

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CLAIMS

1. Avidin dimer, in which two molecules of avidin are bound via the $-NH_2$ groups by means of suberate.

2. Avidin dimer, in which two molecules of avidin are bound via the –COOH groups by means of polyethylene glycol with a molecular weight of 3400.

3. Dimer according to claim 1 or 2, wherein the avidin is streptavidin.

4. Pharmaceutical and/or diagnostic composition containing the dimer of any one of claims 1-3.

5. Composition according to claim 4, which can be administered parenterally or locoregionally.

6. Use of the dimer of any one of claims 1-3 for the preparation of medicaments and diagnostic means.

7. Use of the dimer of any one of claims 1-3 for the preparation of a medicament useful in the diagnosis or treatment of pathological conditions of organs or tissues.

8. Use of the dimer of any one of claims 1-3 for the preparation of medicaments or diagnostic means useful for the therapy or diagnosis of tumours.

9. Use of the dimer of any one of claims 1-3 in pretargeting methods using antibodies *in vitro*.

10. Use of the dimer of any one of claims 1-3 for the preparation of a medicament useful for the treatment of disease using pretargeting methods with antibodies.

11. Use according to claim 10, where said disease is a tumour.

12. Use according to claim 10, where said antibody is an anti-tenascin antibody.

13. Use according to claim 12, where said anti-tenascin antibody is monoclonal.

14. Use according to claim 10, where said medicament is part of a kit which is useful in the diagnosis and treatment of tumours by means of the three-step pretargeting technique.

15. Use according to claim 14, where said kit contains a radiopharmaceutical.

16. Kit for the radiotherapy or diagnosis of tumours, characterised in that at least one of the components of said kit contains a dimer according to claim 1 or claim 2.

17. Kit according to claim 16, for use in the pretargeting technique.

18. Kit according to claim 17, where said pretargeting technique is three-step.

19. Kit according to any one of claims 16, 17 or 18, containing a biotinylated anti-tenascin.

20. Kit according to claim 19, where said antibody is a monoclonal antibody.

FIGURE 1

Separation of Diavidin 1, 2 and 3 by gel filtration

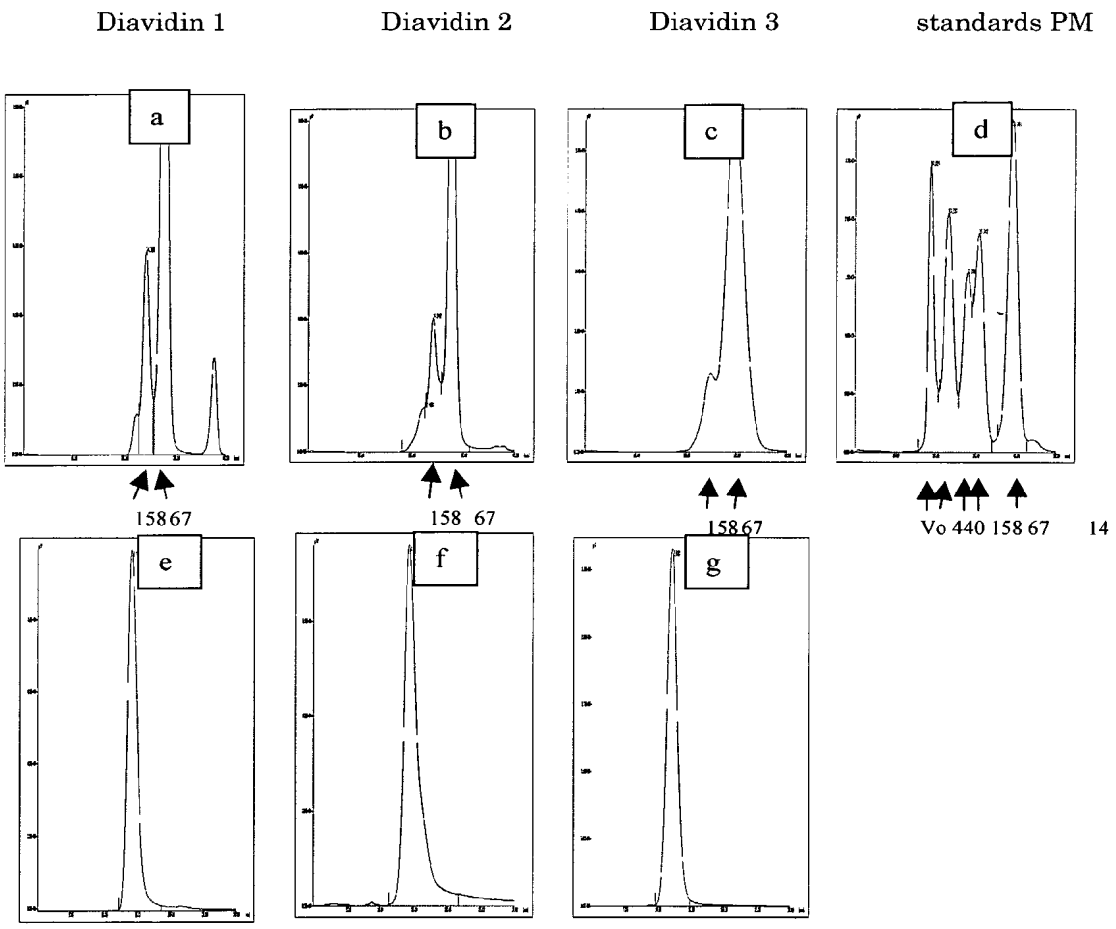
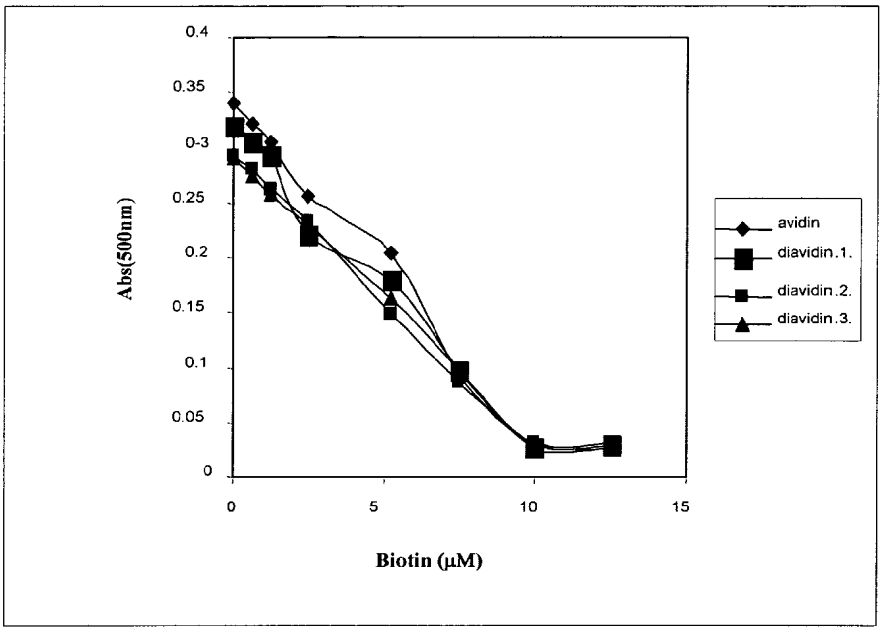


FIGURE 2


0.4
0.35
0-3
0.25
0.2
0.15
0.1
0.05
0
Abs(500nm)
0
5
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15
Biotin (µM)
avidin
diavidin.1.
diavidin.2.
diavidin.3.

FIGURE 3

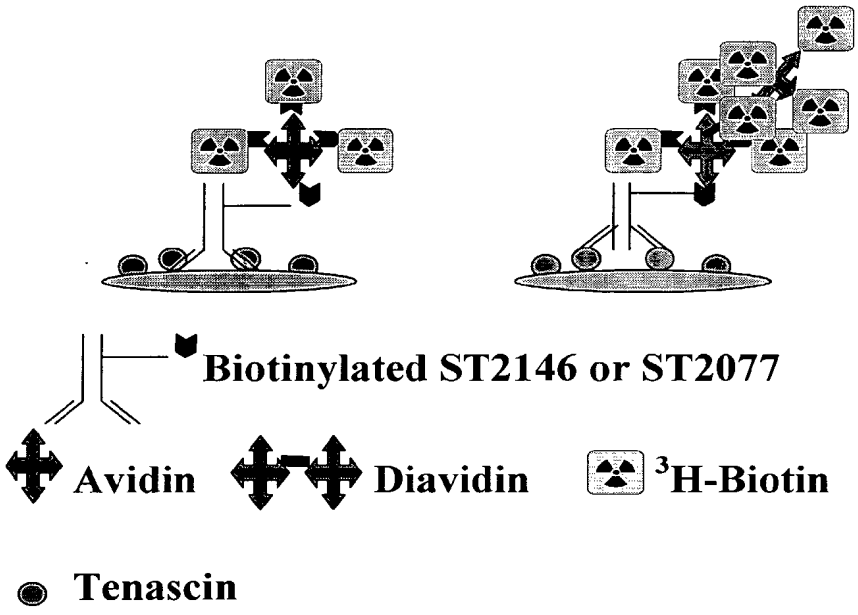
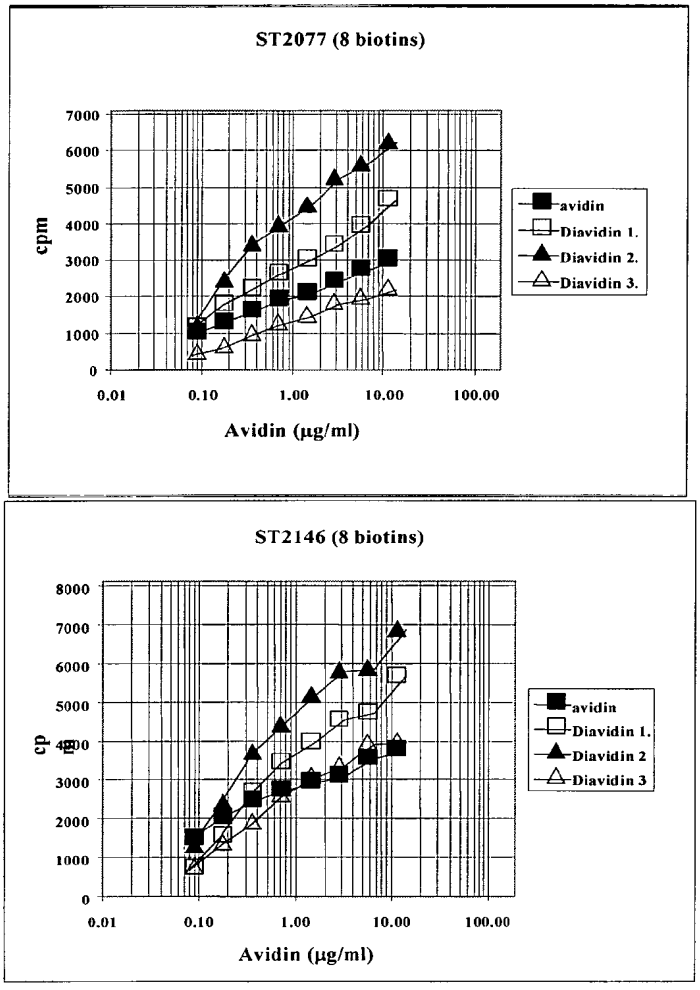

Biotinylated ST2146 or ST2077
Avidin
Diavidin
^{3}H-Biotin
Tenascin

FIGURE 4


ST2077 (8 biotins)
cpm
7000
6000
5000
4000
3000
2000
1000
0
0.01
0.10
1.00
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100.00
Avidin (µg/ml)
avidin
Diavidin 1.
Diavidin 2.
Diavidin 3.
ST2146 (8 biotins)
cp
8000
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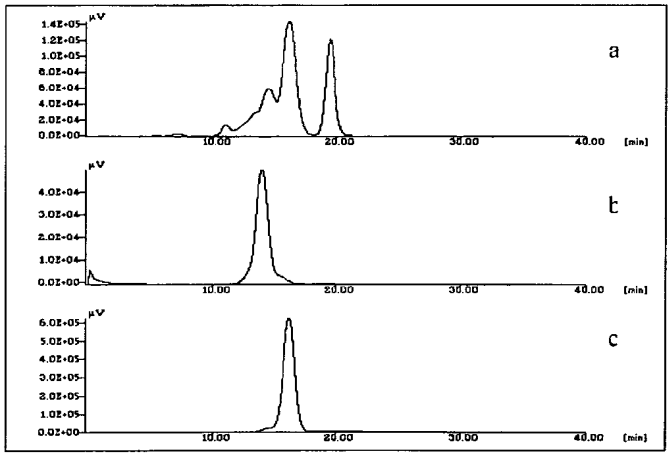
Avidin (µg/ml)
avidin
Diavidin 1.
Diavidin 2
Diavidin 3

FIGURE 5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[min]
a
μV
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3.0E+04
2.0E+04
1.0E+04
0.0E+00
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20.00
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40.00
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5.0E+05
4.0E+05
3.0E+05
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40.00
[min]
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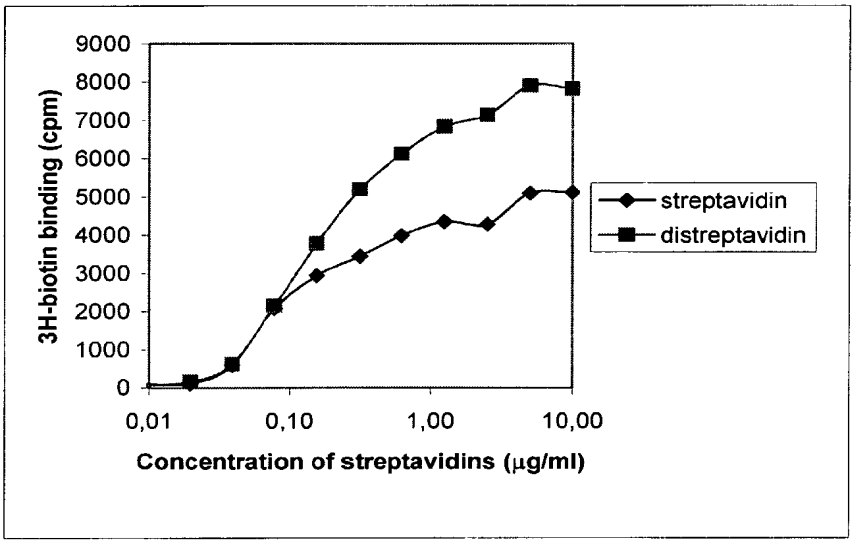
FIGURE 6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4000
3000
2000
1000
0
3H-biotin binding (cpm)
0,01
0,10
1,00
10,00
Concentration of streptavidins (µg/ml)
streptavidin
distreptavidin

INTERNATIONAL SEARCH REPORT

Inter I Application No
PCT/IT 03/00135

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48 A61K51/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 482 698 A (GRIFFITHS GARY L) 9 January 1996 (1996-01-09) cited in the application examples 5,14,15 ---	1-20
A	CHINOL M ET AL: "Biochemical modifications of avidin improve pharmacokinetics and biodistribution, and reduce immunogenicity." BRITISH JOURNAL OF CANCER, vol. 78, no. 2, July 1998 (1998-07), pages 189-197, XP009012930 ISSN: 0007-0920 cited in the application figure 2; table 2 --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2003	10/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lanzrein, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	REZNIK G O ET AL: "STREPTAVIDINS WITH INTERSUBUNIT CROSSLINKS HAVE ENHANCED STABILITY" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 14, August 1998 (1998-08), pages 1007-1011, XP002926127 ISSN: 1087-0156 ---	
A	CHINOL M ET AL: "BIODISTRIBUTION IN TUMOUR-BEARING MICE OF TWO 90Y-LABELLED BIOTINS USING THREE-STEP TUMOUR TARGETING" NUCLEAR MEDICINE COMMUNICATIONS, XX, XX, vol. 18, no. 2, February 1997 (1997-02), pages 176-182, XP008008175 ISSN: 0143-3636 ---	
A	CREMONESI M ET AL: "THREE-STEP RADIOIMMUNOTHERAPY WITH YTTRIUM-90 BIOTIN: DOSIMETRY AND PHARMACOKINETICS IN CANCER PATIENTS" EUROPEAN JOURNAL OF NUCLEAR MEDICINE, BERLIN, DE, vol. 26, no. 2, February 1999 (1999-02), pages 110-120, XP000984147 ISSN: 0340-6997 cited in the application ---	
A	HART P R ET AL: "HPMA COPOLYMER-MODIFIED AVIDIN: IMMUNE RESPONSE" JOURNAL OF BIOMATERIALS SCIENCE. POLYMER EDITION, VSP, UTRECHT, NL, vol. 11, no. 1, 2000, pages 1-12, XP008005603 ISSN: 0920-5063 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IT 03/00135

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 6-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all *searchable claims*.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr al Application No
PCT/IT 03/00135

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5482698 A	09-01-1996	AU 674580 B2	02-01-1997
		AU 6666694 A	08-11-1994
		CA 2161109 A1	27-10-1994
		EP 0695194 A1	07-02-1996
		JP 8509226 T	01-10-1996
		SG 46472 A1	20-02-1998
		WO 9423759 A1	27-10-1994